Association of Fecal Coliforms With Soil Aggregates: Effect of Water Content and Bovine Manure Application

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Abstract: Manure-borne microorganisms and organic particles can move in soils in large interconnected pores or in long macropores. These pathways in structured soils are located between soil aggregates or peds. Therefore, interaction with soil aggregates may substantially affect the ability of macropores to serve as bacterial conduits.

The study tested the hypothesis that fecal coliform (FC) association with soil aggregates is affected by aggregate size, water content, and bovine manure application.

Tyler loam soil aggregates were separated into three fractions. Air-dried and water-saturated aggregates were submerged in water-FC and water-manure-FC suspensions with concentrations of 10^3, 10^4, or 10^5 CFU mL^{-1} for 24 h. The maximum association of FC with aggregates was observed in the experiments with air-dried aggregates in the water-FC suspensions; no measurable FC amount was associated with the saturated aggregates in the same suspension. In the water-manure-FC suspension, about 2.5 times more FC were associated with the air-dried soil aggregates than with the water-saturated aggregates. The FC amount associated with air-dried aggregates in the water-manure-FC suspension was about 300 times less compared with the amount in the water-FC suspension. The FC association with the aggregates was not affected by aggregate size. Increased water content of soil aggregates and presence of manure in water-FC suspensions decreased FC association with soil aggregates. Because FC transport in soil generally occurs through interaggregate pores after rainfalls after manure applications, a decrease in bacteria-soil association with aggregates can enhance bacterial mobility and increase risk of groundwater contamination.

Key words: Soil aggregates, bovine manure, fecal coliforms, water content.

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Manure is the primary source of bacterial groundwater contamination in agricultural settings (Jamieson et al., 2002; Ferguson et al., 2003; Pachepsky et al., 2006). Rainfall creates suspensions of bacteria (including pathogenic strains) and manure particulates, which then enter soil with infiltration flow and may be transported to groundwater. Because of their size, bacteria and manure particles can move in soils in large interconnected pores or in long macropores (Bitton et al., 1974; Wollum and Cassel, 1978; Smith et al., 1985; McMurry et al., 1998). Under such conditions, the average bacterial velocity in soil can be larger than the average soil water velocity because bacteria are transported with water only in a part of soil pore space where water velocity is larger than average (Hagedorn et al., 1978; Natsch et al. 1996). Unc and Goss (2003) found that the velocity of bacteria was 35 times greater than the average pore-water velocity in soil in the vertical transport of coliforms from liquid swine and solid cow manure applied on the surface of silty loam and sandy loam soils. Guber et al. (2005a) observed a 30-fold difference between bacterial and average velocities when they applied bacteria-manure suspensions on soil columns containing macropores.

Soil primary particles of sand, silt, and clay sizes are spatially arranged into larger units of soil structure that, depending on their size, are called peds or aggregates. Large pores such as macropores are located between these large structural units. Therefore, bacterial interaction with soil aggregates may substantially affect the ability of macropores to serve as bacterial conduits.

Previous experiments on bacterial attachment to soil were carried out with soil sieved through fine mesh or with soil primary particles of clay, silt, or sand (Weaver et al., 1978; Ling et al., 2002). The authors observed an increase in adherence of bacteria to soil with the increase of clay content. Those experiments were conducted with manure-free suspension. Guber et al. (2005b) showed that presence of manure in the suspension significantly decreased *Escherichia coli* attachment to loam and sandy clay loam soil particles.

Although such studies have been instrumental in elucidating mechanisms of attachment, they are of limited relevance in understanding and predicting bacterial interaction with soil aggregates. In this study, we define the bacterial association with aggregates as the result of mechanisms by which bacteria are removed from large interaggregate pores to stay within or on the surface of aggregates. Bacteria can be associated with a soil aggregate not only by attachment or adhesion to the internal and external surfaces, but also by residing in the aggregate pore space after being transported inside the aggregate by the capillary flow or because of motility. The importance of the latter mechanism should depend on aggregate soil water content that, in turn, is controlled by the history of rainfall events and evaporation; soil aggregates can have different degrees of water saturation, from almost dry to fully saturated. Although concurrent infiltration of dissolved manure material and microorganisms in structured soils is common, we are not aware of previous studies of fecal coliform (FC) association with soil aggregates and manure effect on it.

The objective of this work was to investigate the effect of soil aggregate size, antecedent aggregate water content, and the presence/absence of manure on FC association with soil aggregates.

MATERIALS AND METHODS

Soil samples were taken from the A horizon of Tyler loam soil (fine-silty, mixed, mesic, Aeric Fragiaquults) in Franklin County, PA. The soil was under a mixture of grasses and legumes. Soil was sampled from the top 15-cm soil layer of 50 × 50 cm size plot to minimize spatial variability of aggregate properties. Soil was air dried and sieved through 3/8", 5/16", no. 4, and no. 6 mesh size sieves (Advantech Manufacturing, New Berlin, WI) to obtain aggregate fractions of 3.35 to 4.75 mm, 4.75 to 7.93 mm, and 7.93 to 9.5 mm.

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Organic carbon (OC) content was measured in the aggregate fractions with the dry combustion method (Nelson and Sommers, 1996). Soil texture was measured with the pipette method after dispersion with sodium pyrophosphate, $Na_4P_2O_7$ (Gee and Or, 2002). The values of soil pH and electrical conductivity (EC) were measured in the 1:1 paste with the ion-selective electrode and the Solomat MPM 1000 conductivity meter (Solomat Ltd, Bishops Stortford, UK [manufacturers' names are given for information only and do not constitute endorsement by the USDA]), respectively. Selected properties of the soil are given in Table 1.

Bovine manure was collected from the Dairy Research Unit at the US Department of Agriculture–Agricultural Research Service facility in Beltsville, MD. The manure contained 15.8% total solids, 1.21 g L^{-1} of total nitrogen, 0.31 g L^{-1} of ammonium nitrogen, less than 0.001 g L^{-1} of nitrate, 0.15 g L^{-1} of soluble phosphorus, and 0.30 g L^{-1} of total phosphorus. The bovine manure was added to water to obtain water-manure suspensions of 40 g L^{-1} and filtered through cheesecloth to separate the suspension from plant residues and bedding straw. The filtered manure was centrifuged at 100g for 15 min to separate coarse fractions from liquid and colloidal fractions. The particle size distribution of manure colloids was measured using a laser-scattering particle size distribution analyzer LA-920 (Horiba Instruments Inc, Irvine, CA). The average particle size was 2.48 ± 1.48 μm, the solid-liquid ratio was 0.114% ± 0.001%, pH was 6.7, and EC was 1.21 (S m^{-1}) in the centrifuged manure suspension. Fecal coliform concentrations were determined in the manure with a spread plate method (Clesceri et al., 1998). Bacteria-manure suspension in the amount of 50 μL was plated onto MacConkey Agar using an Autoplate 4000 spiral platter (Spiral Biotech, Bethesda, MD) and incubated for 14 h at 44.5 °C. Fecal coliform colony-forming units (CFU) were counted using a Protocol plate reader (Synoptics, Cambridge, UK). The manure suspension was aged for approximately 3 months at 4 °C until FC content in manure decreased to less than 10^3 CFU mL^{-1}.

Aged manure suspension was cultured in dilute yeast extract broth (0.01% wt/vol) at 37 °C for 24 h, with the goal of producing a uniform inoculum for attachment. Cultured cells were pelleted by centrifugal sedimentation at 6000g for 10 min, and the pellet was resuspended in DI water. The cultured cells were added to the water and aged water-manure suspension so as to provide FC approximate concentrations of 10^3, 10^4, 10^5, and 10^6 CFU mL^{-1} in suspensions. The inoculum density was measured before the experiments using the protocol previously described for the manure.

Each soil aggregate fraction was separated into two samples. The first sample remained air dried, whereas the second sample was subjected to gradual saturation for 72 h in water with a pH value of 7.5 and an EC value of 0.08 S m^{-1}. Water contents were measured gravimetrically in all samples in triplicate. Water-FC and water-manure-FC suspensions were added to 3-g air-dried and saturated aggregate samples to obtain soil-to-suspension ratios of 1:10 for each FC concentration in triplicate. The aggregates were kept submerged in water-FC and water-manure-FC suspensions for 24 h at 8 °C to minimize microbial growth. It was determined in preliminary experiments that FC content did not change noticeably in water-FC and water-manure-FC suspensions during 24 h, and that time was sufficient to achieve attachment equilibrium. Fecal coliform concentrations were measured by spiral plating in the water-FC and water-manure-FC suspensions with and without soil aggregates similarly to FC measurement in the manure suspensions described earlier. The amount of FC associated with the soil aggregates was calculated from the difference between the FC amount in suspensions without aggregates and in suspensions with submerged aggregates after all suspensions were kept for 24 h at 8 °C.

Values of pH and EC were measured in applied water-FC, water-manure-FC, and remain suspensions after the aggregate removal using the same instruments as before. Statistical analyses were done using the SPLUS software (Mathsoft, Cambridge, MA).

TABLE 1. Some Physical and Chemical Properties in Aggregate Fractions of the Tyler Loam Soil

Aggregate Size, mm	Clay	Silt	Sand	OC	pH	EC
	%					S m^{-1}
3.35–4.75	16	63	21	2.58	5.62	0.42
4.75–7.93	17	64	19	2.18	5.58	0.39
7.93–9.5	17	64	19	2.63	5.53	0.36

RESULTS

Properties of soil aggregates are summarized in Table 1. Soil texture and OC content did not differ among aggregates of different sizes. A slight decrease in pH and EC values was observed with an increase in aggregate size. Water content was significantly higher in the air-dried aggregates of 3.35 to 4.75 mm as compared with aggregates larger than 4.75 mm. The opposite was observed in the water-saturated aggregates, where water content was higher ($P > 0.95$) in aggregates larger than 4.75 mm. After the air-dried aggregates were submerged, their water content increased but remained smaller than in the gradually saturated aggregates (Table 2). No aggregate slaking was observed during the experiments.

The EC and pH values for water-FC and water-manure-FC suspensions, after attachment experiments, are shown in Fig. 1. The water-manure-FC suspensions had higher pH and EC compared with the water-FC suspensions. Generally, pH was higher in water-FC suspensions with water-saturated aggregates compared with initially air-dried aggregates (Figs. 1A, B). Water-FC suspensions had higher pH with larger aggregates. No statistically significant difference ($P < 0.05$) in pH was observed between water-manure-FC suspensions with air-dried and saturated aggregates (Figs. 1A, B). Water-manure-FC suspensions had slightly higher pH values with aggregates less than 7.93 mm than with aggregates of greater than 7.93 mm. Values of EC in the water-FC suspensions were slightly higher with aid-dry aggregates than with saturated aggregates (Figs. 1C, D). The opposite was true for the water-manure-FC suspensions. No statistically significant difference in EC was found between aggregates of different sizes at the significance level of 0.05.

The FC interactions with soil aggregates were different for the air-dried and saturated aggregates (Figs. 2 and 3). The maximum FC amount associated with air-dried aggregates was found in the water-FC suspension; no measurable FC amount associated with saturated aggregates was found in the same suspension. In the water-manure-FC suspension, about 2.5 times more FC associated with the air-dried soil aggregates than with the water-saturated aggregates. The FC amount associated with air-dried aggregates in the water-manure-FC suspension was about 300 times less compared with amount in the water-FC suspension.

The analysis of variance showed that antecedent water content of aggregates and the presence of manure in water-FC

TABLE 2. Gravimetric Water Content in Soil Aggregates

	Before the Batch Experiment		After the Batch Experiment
	Air-Dried	Water-Saturated	Air-Dried
Aggregate Size, mm	%		
3.35–4.75	4.3 ± 0.10	53.6 ± 0.9	40.3 ± 1.8
4.75–7.93	1.90 ± 0.02	58.2 ± 3.6	50.3 ± 1.4
7.93–9.5	1.58 ± 0.17	58.6 ± 2.4	51.7 ± 1.4

Values are presented as mean ± SD.

suspensions were significant ($P < 0.001$) factors. The effect of aggregate size and initial bacterial concentrations on FC-soil aggregate interaction was not significant at the 0.05 significance level.

DISCUSSION

The antecedent aggregate water content was a significant factor affecting FC association with soil aggregates in the water-FC suspensions. Because FC had an equal opportunity to attach to the outer surfaces of both submerged air-dried and water-saturated aggregates, the differences in amounts of FC associated with aggregates are presumably attributable to differences in intraaggregate surfaces and pore space available for bacteria and/or the differences in numbers of bacteria entering into aggregates. We conjecture that mechanisms of bacterial transport into aggregates are different depending on

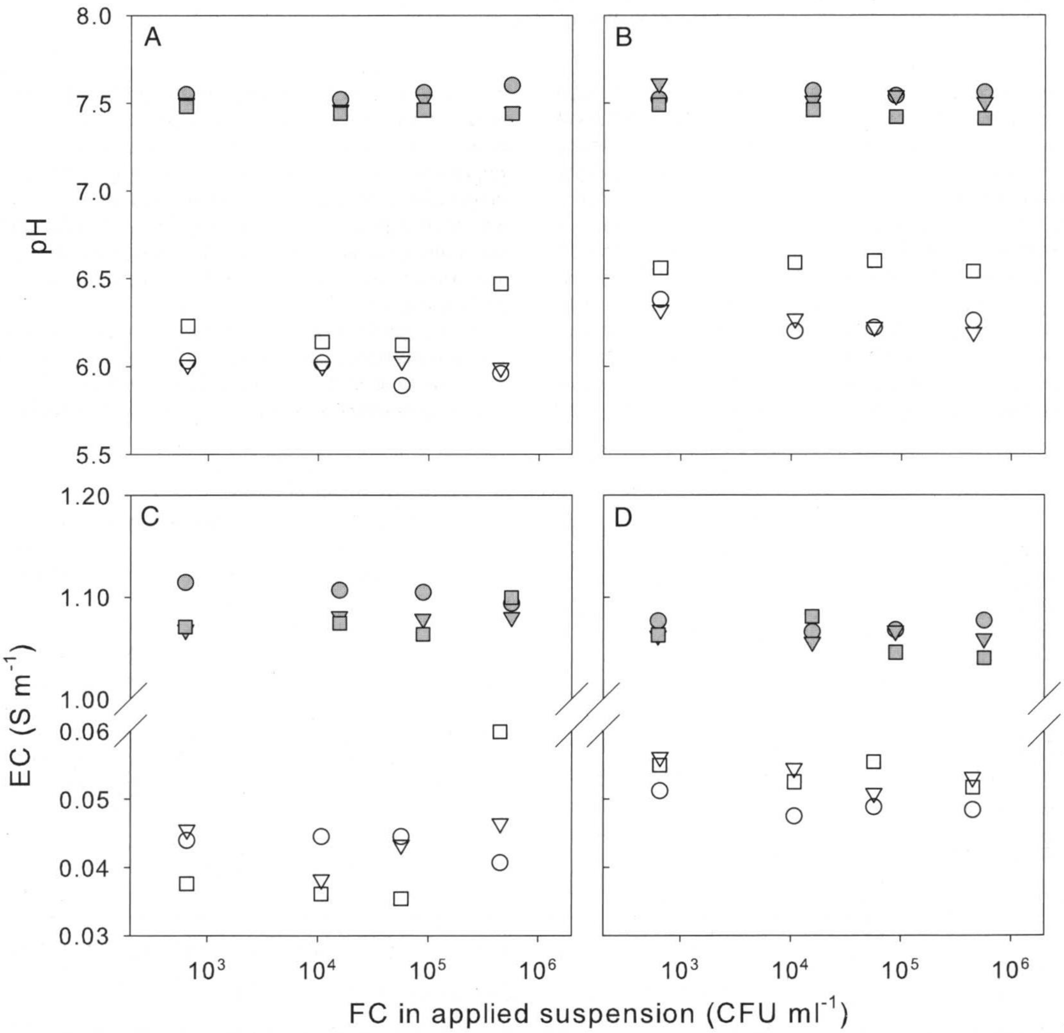

FIG. 1. Values of pH (A, B) and EC (C, D) of water-FC suspensions without (open symbols) and with manure (filled symbols). Air-dried (A, C) and saturated (B, D) aggregates were immersed in the suspensions. Aggregate sizes: (O) 3.35 to 4.75 mm, (▽) 4.75 to 7.925 mm, and (□) 7.925 to 9.5 mm.

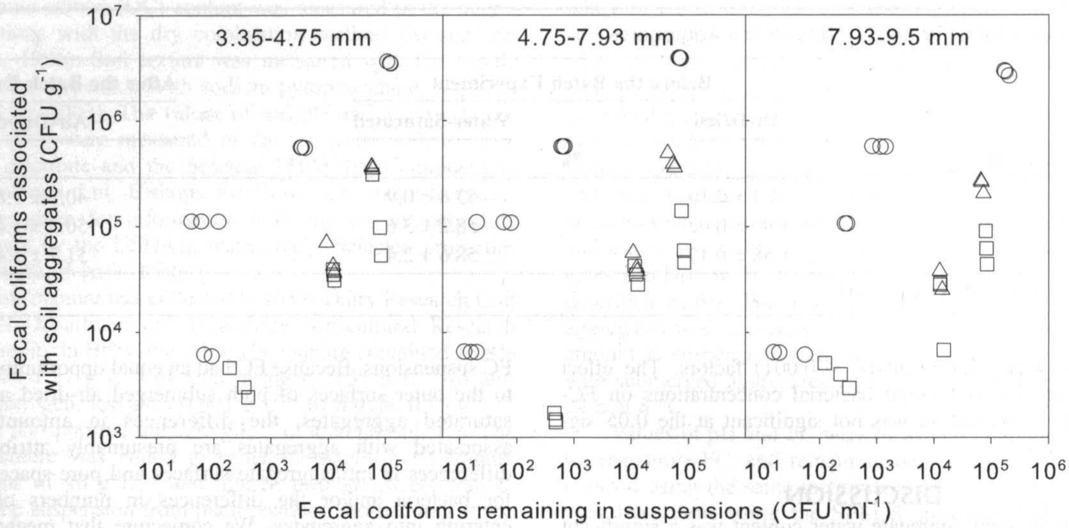

FIG. 2. Fecal coliforms concentrations per unit mass of pore solution in air-dried aggregates in water-FC suspensions (O), air-dried aggregates in water-manure-FC suspensions (Δ), and saturated aggregates in the water-manure-FC suspension (□). Each panel contains data points for four initial bacterial concentrations, unless cell attachment was less than 10^3 CFU g^{-1}. Data are not shown for saturated aggregates in water-FC suspension because of negligible interaction.

antecedent water content. The FC transport into water-saturated aggregates occurs most likely because of Brownian diffusion and chemotaxis, which are relatively slow processes, whereas transport into air-dried aggregates is driven by the capillary pressure gradient between air-dried soil matrix and free water in suspension, which is a relatively rapid process. The differences in bacterial habitats outside and inside aggregates led Hattori and Hattori (1976) and Harris (1994) to suggest that microorganisms partition into interaggregate and intraaggregate fractions. Bundt et al. (2001) found that microbial biomass was more abundant along preferential flow paths than within soil structural units. The opposite has been shown in other studies that used microphotography (Kilbertus, 1980; Foster 1988) and enumeration and DNA fingerprinting (Ranjard et al., 2000). Observation of the soil micromorphology in the work of Fisk et al. (1999) revealed that bacterial adsorption tended to occur predominantly within and along the intergrain matrix. Chenu et al. (2001) found that microbial partitioning between external and internal parts of soil structural units depended on soil texture and water content. Water content, soil solution composition, and soil solid phase properties all have a role to play in bacterial partitioning in soil pore space.

The difference in surfaces available for bacteria-soil interactions may also be a reason for differences in association with air-dried and water-saturated aggregates. Water contents after experiments were less in the air-dried compared with the

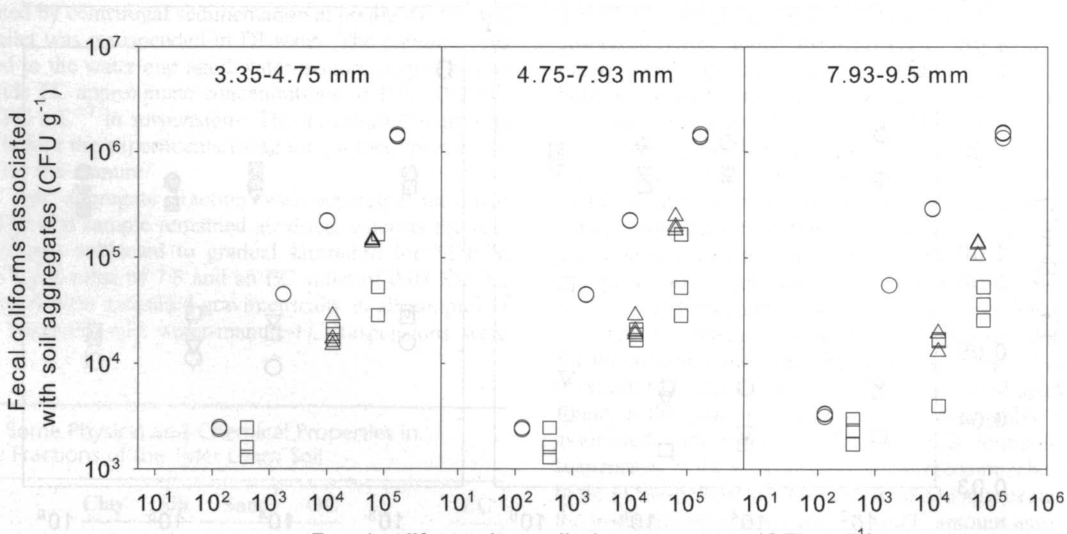

FIG. 3. Fecal coliforms concentrations per unit mass of dry aggregates for air-dried aggregates in water-FC suspensions (O), air-dried aggregates in water-manure-FC suspensions (Δ), and saturated aggregates in the water-manure-FC suspension (□). Each panel contains data points for four initial bacterial concentrations, unless cell attachment was less than 10^3 CFU g^{-1}. Data are not shown for saturated aggregates in water-FC suspension because of negligible interaction.

water-saturated aggregates (Table 2) because of the presence of trapped air. Therefore, air-water interfaces existed inside submerged air-dried aggregates. Strong retention of bacteria by air-water interfaces has been directly observed or indirectly inferred by several authors (Wan et al., 1994; Powelson and Mills, 1996; Schäfer et al., 1998). The presence of trapped air in our experiments, therefore, did not decrease the potential for bacterial retention inside of aggregates because the presence of water films created surfaces even more favorable for FC retention.

The presence of manure substantially diminished FC interaction with air-dried aggregates. This observation is similar to results obtained in *E. coli* attachment experiments to loam and sandy clay loam soil particles (Guber et al., 2005b). Previous studies indicate that an increase in pH (e.g., manure application) typically results in decreased bacterial attachment to mineral surfaces (Bitton et al., 1974; Scholl et al., 1990; Scholl and Harvey, 1992; Kinoshita et al., 1993). The effect of EC is more complex; at low ionic strengths, bacterial attachment may increase with increasing EC (Mills et al., 1994; Lin et al., 2003), whereas at higher ionic strengths, attachment may decrease with increasing EC (Gordon and Millero, 1984). However, all of these studies were conducted with suspended soil particles and, therefore, may not be directly applicable to soil aggregates. Most of previous studies also did not involve the complex solution species present in manure, so the presence of manure might have larger potential impact than caused by changes in pH and EC values in suspension. A potential explanation for the diminished FC interaction with air-dried aggregates observed with manure application may be the clogging of intraaggregate fine pores by manure colloids and/or competition with other manure-borne organisms for attachment sites.

Changes in the effects of aggregate water content and presence of manure on the bacterial association with aggregates should be expected if temperatures vary. There are reports that temperature increases in the range of 4 °C to 35 °C cause increases in bacterial adhesion to surfaces (An and Friedman, 2000), motility (Maeda et al., 1976; Macnab, 1996), and capillary infiltration rates into dry soils according to the temperature dependence of the kinematic viscosity and of the air-liquid surface tension (Zhang et al., 2003; Smiles, 2005).

The analysis of variance test did not reveal differences in FC amounts associated with aggregates of different size. Aggregate fractions had particle size distribution and organic matter content (Table 1). Differences in pH (Fig. 1), albeit statistically significant, were probably not sufficiently large to substantially change the association. It remains to be seen whether some discernible differences in aggregate textural composition and/or OC content will affect the FC interactions with aggregates.

The increased amount of FC associated with dry aggregates implies an interesting interplay between the soil water content before rainfall and transport of manure-borne bacteria with infiltrating rainfall water. Because infiltration rates are much higher when soil is dry, the interaction of bacteria with dry aggregates may be also enhanced. However, the rapid entry of bacteria into soil does not guarantee rapid downward transport because they may be retained in dry topsoil aggregates. As the soil becomes wetter, bacterial interaction with aggregates may decrease resulting in enhanced downward transport with macropore flow. Simultaneously, rates of manure dissolution may impact bacterial transport by directly affecting interaction with aggregates. Consequently, the effect of initial soil water saturation on manure-borne bacterial transport presents an interesting topic for further exploration.

ABBREVIATIONS

BARC: Beltsville Agricultural Research Center;
CFU: colony-forming units.

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